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**HSM-LUP-937**

The European Patent Office  
D-80298, Munich  
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15 October 2004

Kind Attn: Ms. Monica Ferro  
Authorised Officer

BY COURIER

Dear Sirs,

Re: LUPIN LIMITED *et al.*  
PCT International Application No. PCT/IN03/000345  
Filed on: 27 October 2003  
**RESPONSE TO FIRST WRITTEN OPINION**  
**DUE 05 November 2004**

A response to the **FIRST** Written Opinion is enclosed herewith. The response comprises:

1. A reply regarding novelty and inventiveness of the present invention;

It is respectfully submitted that the response submitted herewith clearly establishes novelty and inventiveness.

It is respectfully requested that the response is duly considered and a favourable International Preliminary Examination is established.

Yours truly

  
H. Subramaniam

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PCT/IN03/00345

Applicants' file reference: HSM-LUP-937

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PCT International Application  
No. PCT/IN03/00345

Applicant: LUPIN LIMITED *et al.*

§ HSM-LUP-937

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Authorised Officer: Ms. Monica Ferro

27 October 2003.

Enclosed with this response are the following:

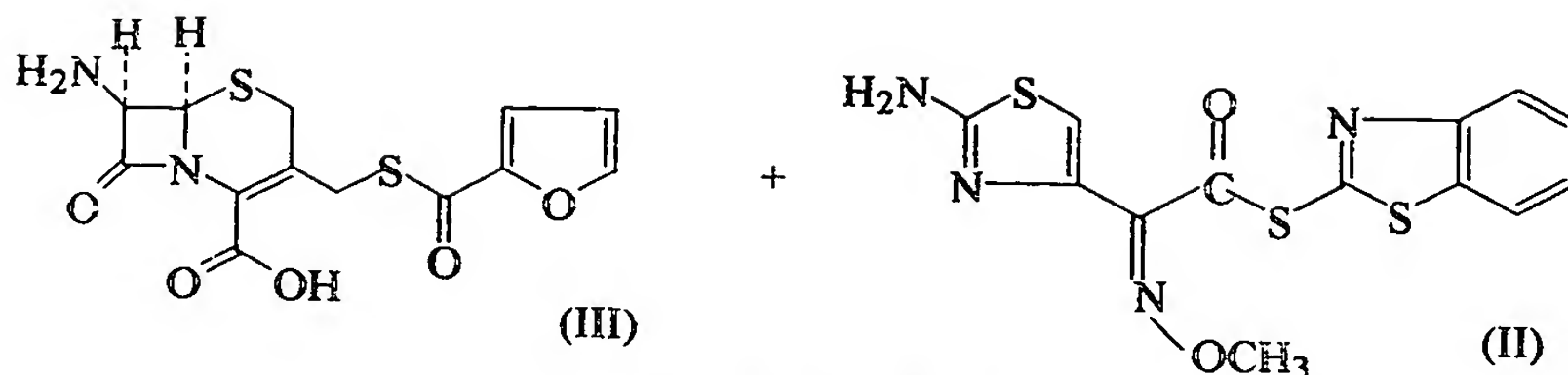
1. A reply regarding novelty and inventiveness of the present invention;

Reference is now made to the first written opinion mailed us on 05 August 2004. The applicants are grateful to the learned Examiner for granting us two months extension of time to file a response.

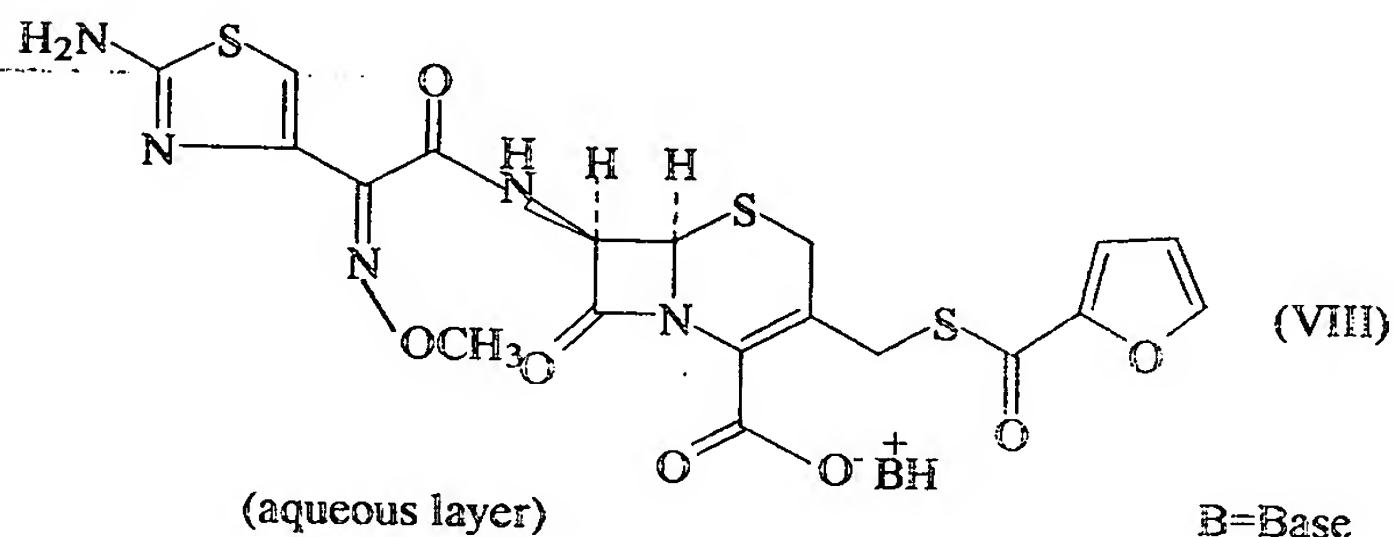
The Applicants note that the International Search Report has cited two documents in the "X" category. The first document has been cited against claims 1-18 of the present application, and the other document has been cited against claim 19 of the present application. Both these documents collectively question the novelty of the invention embodied in the present application.

The applicants have carefully studied both the cited documents and respectfully submit that the method embodied in PCT Application No. PCT/IN03/00345 is both novel and inventive over the methods disclosed in the documents cited.

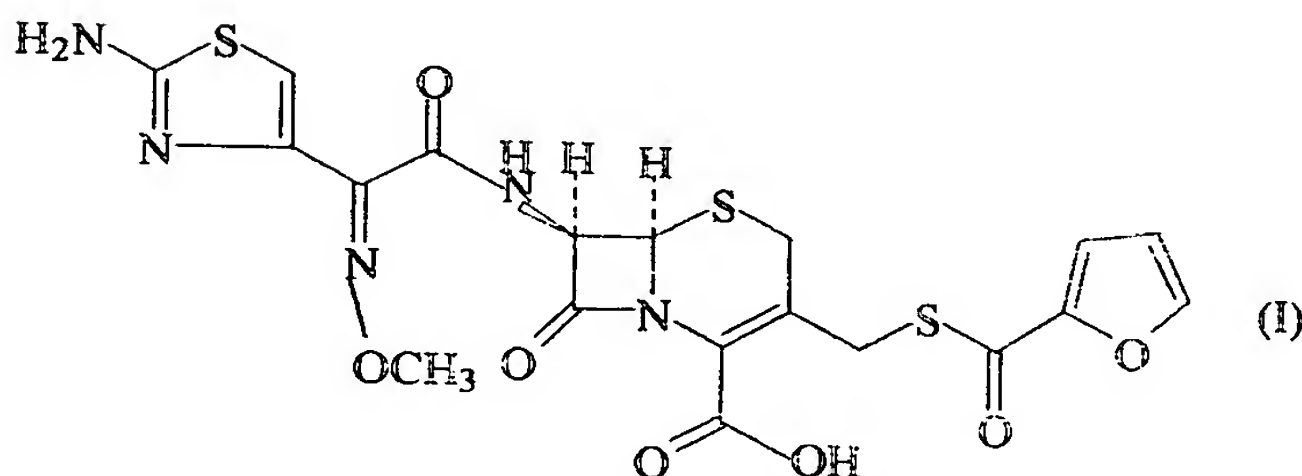
I. At the outset, it is respectfully submitted that the applicants' invention relates to an improved method for preparation of ceftiofur acid (I), comprising reaction of [2-(2-aminothiazol-4-yl)]-2-syn-methoxyimino acetic acid -2-benzothiazolyl thioester of formula (II) and 7-amino-3-thiofuraylmethyl-3-cephem-4-carboxylic acid of formula (III) in a mixture of dichloromethane/water, and in the presence of an organic base (as shown below)



- (i) mixture of a water-immiscible organic solvent and water  
(ii) Base, 0-30°C  
(iii) Quenching with water  
(iv) Separation of aqueous phase,



- i)  $H^+$ , water-miscible and water immiscible organic solvents, and saturated aqueous solution of an alkali or alkaline earth metal salt,  
ii) separation of organic layer,  
iii) evaporation of organic solvent or precipitation with a co-solvent,  
iv) filtration and drying at 35-40°C.



at a temperature between 0-30°C and isolating ceftiofur by crystallization from a mixture of organic solvents to give ceftiofur of formula (I) in high purity and substantially free of impurities.

The invention residing in the applicants' application No. PCT Application No. PCT/IN03/00345 (WO 04/039811 A2) lies

- i) in the manner in which acylation of 7-amino-3-thiofuroylmethyl-3-cephem-4-carboxylic acid (III) with 2-(2-aminothiazol-4-yl)-2-syn-methoxyimino acetic acid-2-benzothiazolyl ester (II) is achieved, and
- ii) in the isolation method, which preferentially removes impurities during the process of acidification in presence of saturated brine solution and also during the final

isolation of ceftiofur (I) utilizing a mixture of a polar and a non-polar organic solvent.

The utilization of 2-(2-aminothiazol-4-yl)-2-syn-methoxyimino acetic acid-2-benzothiazolyl ester (II), for the said acylation reaction is not novel as it was reported earlier in EP 0 037 380 and later patents which disclose improved methods for acylation. This fact has been acknowledged, on page 6, lines 26-29 and page 7, lines 1-11 of our application.

Further, the method disclosed in the applicants' application No. PCT/IN03/00345 (WO 04/039811 A2) is a selection of

- a) solvent employed,
- b) choice of protic solvents,
- c) amount of protic solvent.

Firstly, the present inventors found that chlorinated solvent especially dichloromethane was found to be much better solvent than an alkyl acetate like ethyl acetate, which would be evident from the results tabulated in Table-I, herein for the learned Examiner's ready reference.

*Table-I: Effect of solvent on product conversion, impurity formation, yield and purity of ceftiofur.*

No.	Reaction details	Effect of solvent on reaction		Remarks
		Dichloromethane/ water	Ethyl acetate/water	
1.	Reaction time (hours)	90-100 minutes	300 minutes	Reaction employing dichloromethane / water combination is superior to ethyl acetate / water combination in terms of shorter reaction time, better conversion, lower impurity formation, yield and high purity.
2.	Ceftiofur conversion (%)	95-96	41% unreacted : 6.52%	
3.	Total Impurity after isolation (%)	2.0 to 4.0	34.0	
4.	Yield (%)	65%	15.38%	
5.	Purity (%)	96-98	66.01%	

Further, the reactants are not completely soluble in ethyl acetate/water combination. Therefore, the reactants separate out as a sticky mass, which remains through the reaction thereby increasing the reaction time, impurity formation and making the isolation process quite tedious.

Secondly, the attention of the Learned Examiner is invited to page 18 Table-II of applicants' application No. PCT/IN03/00345 (WO 04/039811 A2). The Learned Examiner will note that the rate of the reaction is also dependent on the addition of a protic solvent like water or an alkanol like methanol. The reaction was also found to be affected by the type of the polar solvent added. Water was preferred to methanol due to better product conversion, lower impurity formation as would be evident from Table-II of applicants' application No. PCT/IN03/00345 (WO 04/039811 A2).

The amount of water added was also found to affect the rate of the reaction as would be evident from Table-II, herein below:

*Table-II: Effect of different proportion of dichloromethane/water on the rate of the reaction.*

No.	Proportion of Dichloromethane : water(v/v)	Duration of reaction (minutes)	Product conversion (%)	Total impurities (%)
1.	98.5: 1.5	150	94.0	6.0
2.	97.5: 2.5	90	95-96	4.0-5.0



3.	95.0: 5.0	95-100	95-96	4.0-5.0
Remarks: Best results are obtained when the proportion is between 95.0:5.0 and 97.5:2.5.				
Purity of the isolated ceftiofur (I) is between 96-98%.				

2. The International Search Report of PCT Application No. PCT/IN03/00345 (WO 04/039811 A2) cites two documents in the "X" category viz.

- a) Zhongguo Yiyao Gongye Zazhi 2001, 32 (6), pages 241-242 (relevant to claims 1-18),
- b) US 4 937 330 (relevant to claim 19)

The Chinese journal reference Zhongguo Yiyao Gongye Zazhi 2001, 32 (6), pages 241-242 teaches a method for preparation of ceftiofur comprising reaction of 7-amino-3-methylsubstituted-3-cephem-4-carboxylic acid by employing 2-(2-aminothiazol-4-yl)-2-syn-oxyimino acetic acid-2-benzothiazolyl ester employing dichloromethane as solvent and in the presence of a base such as triethyl amine. After completion of reaction, the reaction mass is quenched with water and the aqueous layer separated. Ceftiofur is obtained by adjusting the pH of the aqueous layer with dilute hydrochloric acid to pH 2.5, followed by crystallization of impure ceftiofur employing acetone as solvent.

It is respectfully submitted that in the cited document, the reaction is carried out in a single solvent viz. dichloromethane and not in a mixture of dichloromethane and water as disclosed in the applicants' application No. PCT/IN03/00345 (WO 04/039811 A2).

The method disclosed in the Chinese journal reference was replicated in identical conditions and it was found that the reaction rate was comparatively slow, impurity formation was higher, and ceftiofur (I) had a lower purity. Further it was quite difficult to purify ceftiofur (I) from acetone according to the method disclosed therein due to the poor solubility of ceftiofur (I) in acetone.

A comparison of both the methods in terms of reaction rate, product conversion, impurity formation and purity of ceftiofur obtained is clearly shown in Table-III herein below:

Table-III: Comparison of methods embodied in applicants' Application No. PCT/IN03/00345 (WO 04/039811 A2) for manufacture of ceftiofur v/s the method disclosed in Zhongguo Yiyao Gongye Zazhi 2001, 32 (6), pages 241-242 for preparation of ceftiofur.

No.	Method embodied in WO 04/039811 A2 for preparation of ceftiofur.	Process disclosed in Zhongguo Yiyao Gongye Zazhi 2001, 32 (6), pages 241-242 for preparation of ceftiofur
1.	Reaction carried out in a mixture of dichloromethane and water (two-phases).	Reaction carried out in dichloromethane (single-phase)
2.	Reaction Profile: a) Reaction time (hours): 1.5 to 1.75. b) Product conversion (%): 95.5 to 96.0. c) Total Impurities (%): 4.0 to 4.5.	Reaction Profile: a) Reaction time: 2.0 to 3.0 hours b) Product conversion: 93-94% c) Level of Impurities: 6.0 to 7.0%
3.	Isolation of Ceftiofur a) A biphasic mixture of acetonitrile/ethyl acetate and water is employed for obtaining ceftiofur (I) by acidification with 25% orthophosphoric acid. b) Ceftiofur thus obtained does not	Isolation of Ceftiofur a) Ceftiofur (I) is obtained from aqueous layer by acidification with hydrochloric acid. (Please note that ceftiofur is obtained from a <u>single phase system and not a biphasic system as disclosed in WO 04/039811 A2</u> ). b) Ceftiofur (I) thus obtained

	<p><u>precipitate but remains dissolved in the organic layer.</u></p> <p>c) <u>Purification during isolation:</u> Biphasic mixture containing ceftiofur (I) in organic layer is agitated with saturated sodium chloride solution to remove impurities. [Associated impurities are selectively partitioned into the aqueous layer with pure ceftiofur in the organic layer].  <u>Further purification during isolation:</u> The organic layer is partially concentrated and a non-polar solvent such as cyclohexane is added to separate ceftiofur (I), which is then filtered. [partitioning of impurities between polar and non-polar solvent takes place with the impurities dissolving in the polar or non-polar solvent].</p> <p>d) <i>Purification of ceftiofur not required after isolation</i></p>	<p><u>precipitates out from the aqueous layer and does not remain dissolved in solution.</u></p> <p>c) There is <u>no inbuilt purification method</u> during isolation of ceftiofur</p> <p>d) <i>Purification of ceftiofur required after isolation.</i>  Ceftiofur thus isolated is purified by recrystallization, employing acetone as a solvent.</p>
4.	Purity of ceftiofur (I): 96-98%	Purity: 91-92 % (Note: Purity of ceftiofur (I) is low even after purification)
5.	Total impurities: 2.0 to 4.0%	Total impurities: 8.0 to 9.0%.

It is respectfully submitted that it is quite evident from Table-III that there is substantial difference between the method disclosed in the Chinese reference *Zhongguo Yiyao Gongye Zazhi* 2001, 32 (6), pages 241-242 and the method embodied in applicants' Application No. PCT/IN03/00345 (WO 04/039811 A2).

Further, the method embodied in applicants' Application No. PCT/IN03/00345 (WO 04/039811 A2) is far superior in terms of:

- i) product conversion,
- ii) formation of impurities during reaction,
- iii) isolation method, and
- iv) purity of ceftiofur.

In short, the method embodied in applicants' Application No. PCT/IN03/00345 (WO 04/039811 A2) is materially different from and novel and inventive over the prior art methods for preparation of ceftiofur (I).

It is respectfully submitted that the invention embodied in the applicants' application No. PCT/IN03/00345 (WO 04/039811 A2) is neither taught nor motivated from the teachings of the cited art. There is nothing in the cited art, which suggests that acylation of 7-amino-3-thiofuroylmethyl-3-cephem-4-carboxylic acid with 2-(2-aminothiazol-4-yl)-2-oxymino acetic acid-2-benzothiazolyl ester (II) could be carried out in a biphasic system to give lower level of impurities and also that ceftiofur (I) can be isolated through an isolation method, which selectively partitions the impurities to give ceftiofur(I) of high purity.

- b) US 4 937 330 (assigned to M/S. The Upjohn company; filed on July 31, 1986; derived from a PCT Application WO 87/01117; issued on June 26, 1990) is another document cited in the International Search Report relevant against claim 19 of applicants' application No. PCT/IN03/00345 (WO 04/039811 A2).

This patent teaches a method for the preparation of ceftiofur alkali salts comprising of neutralizing the hydrohalide salt of ceftiofur in an aqueous organic solvent (aqueous tetrahydrofuran) by treating with a basic resin like polyvinylpyridine, filtering the solution to remove the basic resin followed by treatment of the filtrate with the base of an alkali earth metal like sodium-2-ethyl hexanoate to give ceftiofur sodium. The process is briefly summarized in Figure 1 for ready reference.

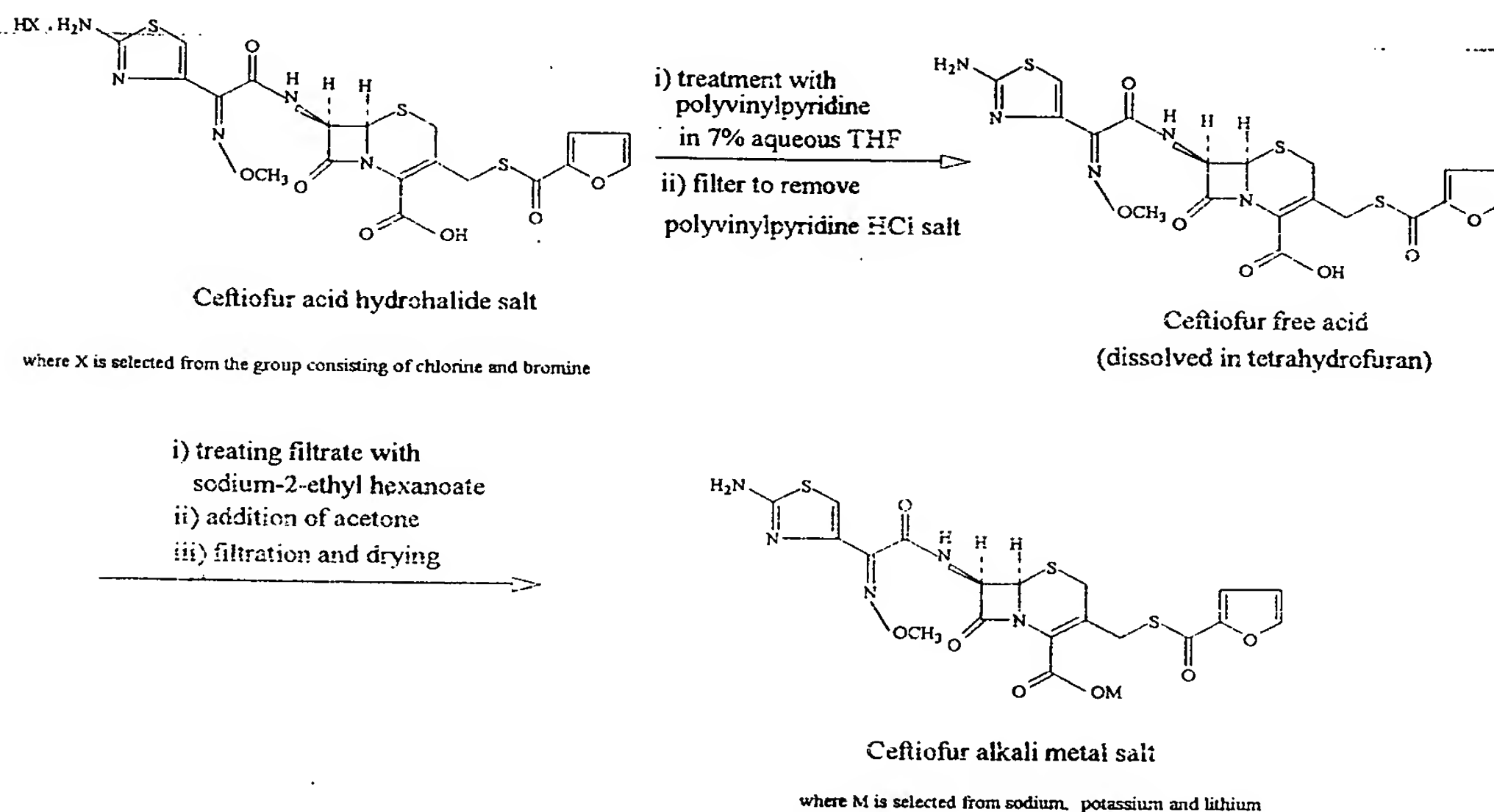


Figure 1: Method as embodied in US Patent No 4 937 330 for preparation of ceftiofur sodium

The method embodied in applicants' the Application No. PCT/IN03/00345 (WO 04/039811 A2) for preparation of ceftiofur sodium is distinct from the method disclosed and claimed in US 4 937 330 since our method (as disclosed in Figure 2 herein below) utilizes ceftiofur free acid and not ceftiofur hydrochloride salt for preparation of ceftiofur sodium.



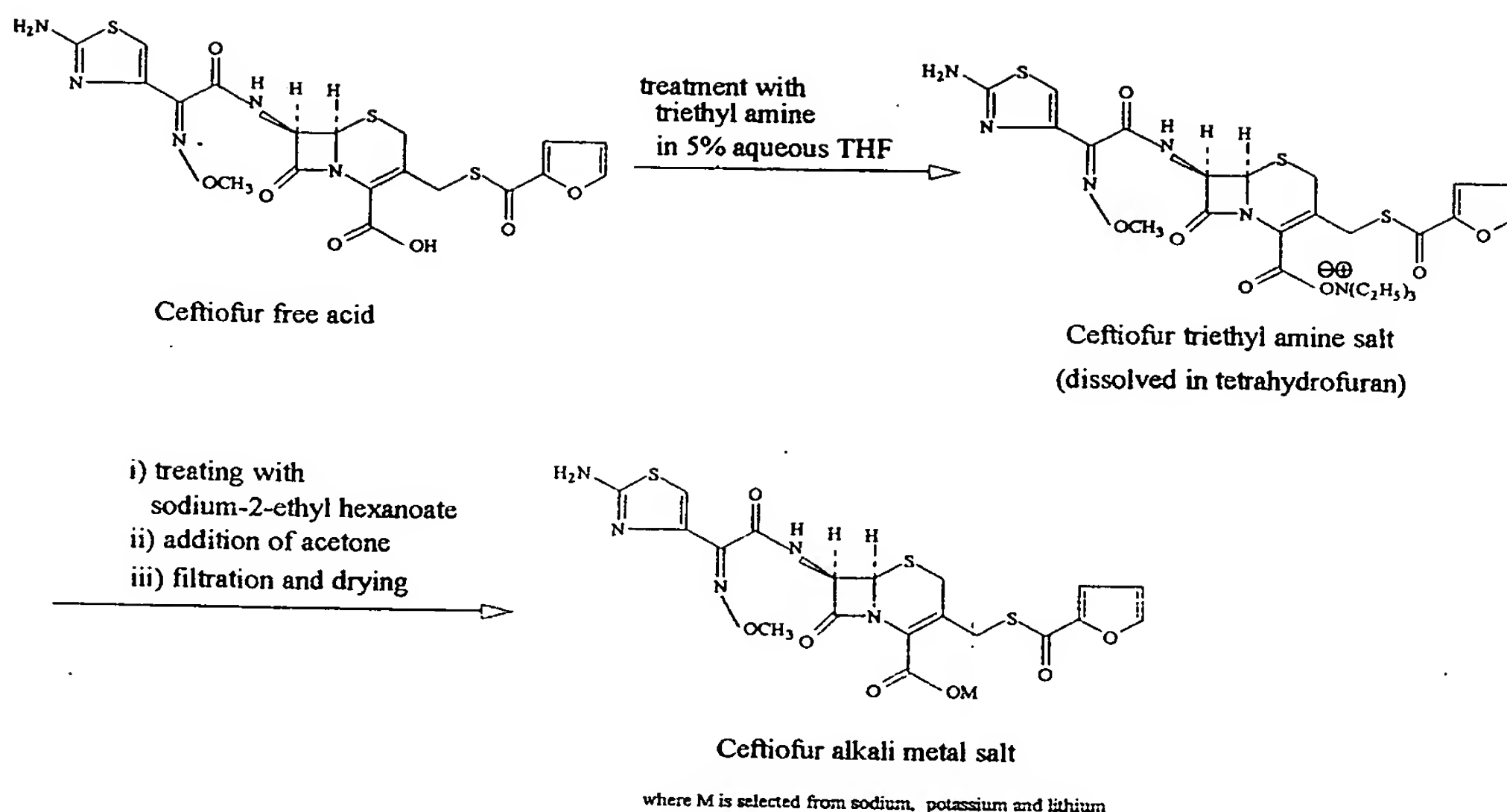


Figure 2: Method as embodied in Lupin's PCT Application No. WO 04/039811 A2 for preparation of ceftiofur sodium

Also, the method embodied in applicants' the Application No. PCT/IN03/00345 (WO 04/039811 A2) is materially different, since this method proceeds through the intermediary of a salt with an organic amine and there is no formation of a ceftiofur hydrohalide salt at any stage.

The distinct differences between the two methods are summarized in Table-IV, for ready reference.

Table-IV: Difference between the methods recited in claim 19 of WO 04/039811 A2 and that embodied in US 4 937 330 for preparation of ceftiofur sodium.

No.	Method as embodied in claim 19 of PCT Application No. WO 04/039811 A2	Method as embodied in US 4 937 330 for preparation of ceftiofur sodium
1.	Ceftiofur free acid is employed for preparing ceftiofur sodium and <u>not ceftiofur hydrohalide salt</u>	Ceftiofur hydrohalide salt and not ceftiofur free acid is employed.
2.	Ceftiofur free acid is converted to its triethyl amine salt and not hydrohalide salt	Ceftiofur hydrohalide salt is neutralized with a basic resin such as polyvinylpyridine to get ceftiofur free acid. Please note there is no formation of an intermediary triethyl amine salt before preparation of ceftiofur sodium..
3.	Ceftiofur triethyl amine salt ( <u>not ceftiofur free acid</u> ) is treated with sodium-2-ethyl hexanoate to give ceftiofur sodium.	Ceftiofur free acid is treated directly with sodium-2-ethyl hexanoate to give ceftiofur sodium

From Table-IV, it would be quite clear that there is a distinct difference between the method disclosed in applicants' Application No. PCT/IN03/00345 (WO 04/039811 A2) and US 4 937 330 for preparation of cefitofur sodium.

### 3. CONCLUSIONS

For the reasons mentioned above, it is respectfully submitted that the invention residing in the applicants' Application No. PCT Application No. PCT/IN03/00345 (published as WO 04/039811 A2) cannot be considered to lack an inventive step and further is neither taught nor motivated from the teachings of prior art. There is nothing in the cited documents, viz.

- i) Zhongguo Yiyao Gongye Zazhi 2001, 32 (6), pages 241-242, and
- ii) US 4 937 330.

which suggests that such an acylation method coupled with an in-built purification method could be contemplated wherein cefitofur acid (I) is obtained in high purity substantially free from impurities.

In view of the submissions made above, we respectfully request that a favourable International Preliminary Examination Report is issued.

Thanking you,

Yours sincerely,

  
Hariharan Subramaniam